Cytokines: **Shared receptors, distinct functions**

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That the signal transduction pathways used by the cytokines IL-2 and IL-15 are identical would suggest that these cytokines have redundant roles in lymphoid development; instead, IL-2 is the guardian of thymus-derived T-cell homeostasis, while interleukin-15 promotes extrathymic development of T and NK cells.

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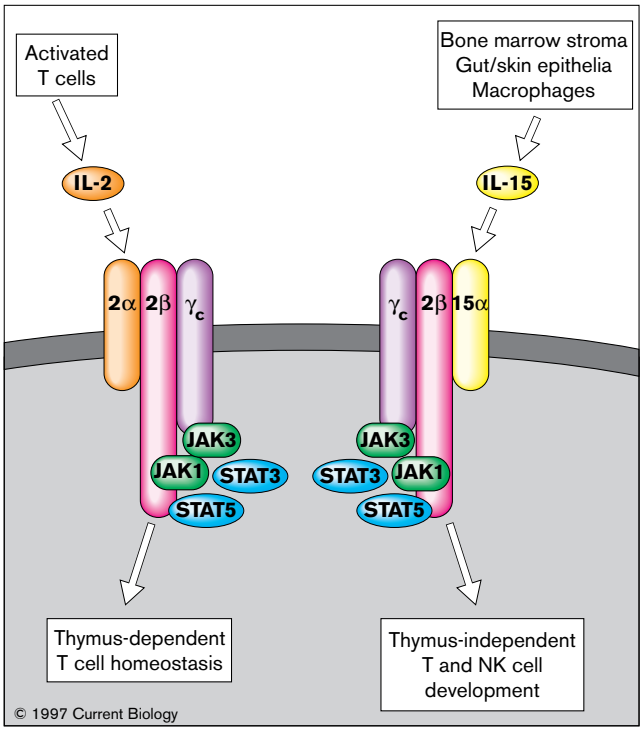
The cytokine road map for lymphopoiesis is often a difficult one to navigate. This is partly due to the fact that cytokines function in myriad fashions, being involved in the survival, proliferation, differentiation and homeostasis of lymphoid cells. Moreover, these soluble mediators are a critical means of communication for effective immune responses, being used by lymphocytes to signal both to each other and to non-lymphoid cells, such as macrophages, epithelial cells and stromal elements. The spectrum of cytokine activity on various subsets of lymphoid cells has been classically defined using *in vitro* assays. However, defining the relevant biological consequences of cytokine interactions with their specific receptors *in vivo* remains a challenge.

The Janus family of tyrosine kinases (JAKs) and the 'signal transducers and activators of transcription' (STATs) are intracellular proteins that are sequentially activated during cytokine receptor signaling. Because a large number of cytokine receptors (more than 25) signal via the somewhat limited panel of JAKs (four known) and STATs (seven known), one major unresolved question is how cytokine responses achieve their final biological specificity. Cytokine responses could in theory be made specific by each cytokine having its own unique receptor. It appears, however, that the receptors for many cytokines have instead evolved towards a situation in which particular receptor chains — such as the gp130 molecule, or the common β and γ chains, β_c and γ_c — are shared amongst different receptor complexes. In principle, this approach would appear to limit specificity.

An extreme example of this is provided by the receptors for interleukin-2 (IL-2) and IL-15 (Fig. 1). The IL-2 and IL-15 receptors share two chains, IL-2Rβ and γ_c, both of which are members of the large cytokine receptor superfamily. The IL-2Rβ–γ_c heterodimer forms the core signaling complex, which associates with JAK1 and JAK3, and activates STAT3 and STAT5. The downstream events that follow ligand binding include the activation of nuclear proteins, such as Myc and Fos, and the upregulation of Bcl-2 and Bcl-X_L levels, leading to resistance to apoptotic cell death and either enhanced survival or proliferation [1].

IL-2 and IL-15 also each associate with a unique third chain, IL-2Rα and IL-15Rα, respectively (Fig. 1). The α chains are related and form a family distinct from the cytokine receptor superfamily [2]. Moreover, as the α chains have short cytoplasmic domains, they do not appear to contribute directly to signal transduction. The primary role of the α chains appears to be to aid the formation of high-affinity receptor complexes, which bind ligand under limiting physiological conditions [3]. In the

Figure 1



IL-2 and IL-15 receptors are trimeric complexes that have unique α chains but a shared core consisting of the IL-2Rβ (2β) and γ_c chains, which signal through JAK1 and JAK3 and STAT3 and STAT5. Although this receptor arrangement suggests that IL-2 and IL-15 might have redundant roles, IL-2 is critical for maintaining peripheral homeostasis of thymus-derived T cells, whereas IL-15 promotes extrathymic T and NK cell development. This response specificity is achieved through a combination of differential ligand production sites and regulation of receptor α chain expression.

absence of an α chain, the IL-2Rβ–γ_c dimer can respond to either IL-2 or IL-15, although the affinity of this receptor is lower. Thus, functional IL-2 and IL-15 receptors are trimeric in nature and distinct, whereas the signaling cascades they initiate when activated appear to be identical.

Although IL-2 and IL-15 show no obvious sequence similarities, they stimulate a similar spectrum of biological activities *in vitro* [2,4]. This is perhaps not surprising, considering the components their receptors have in common. The effects of IL-2 and IL-15 have been demonstrated on the three major lymphoid cell types: immunoglobulin-producing B cells, antigen-specific cytotoxic and helper T cells, and natural killer (NK) cells. IL-2 and IL-15 have been shown to stimulate the proliferation of T, B and NK cells, to augment the cytotoxicity of T and NK cells, to increase immunoglobulin secretion by B cells and to provoke release of interferon-γ from T and NK cells. The sole difference between IL-2 and IL-15 in these assays appears to be in the amount of cytokine needed to elicit a response, which may relate to differential expression of the respective IL-2 and IL-15 receptor α chains in the responding cell populations.

Do IL-2 and IL-15 have redundant functions *in vivo*? Such redundancy could be important in allowing an immune response to be sustained under diverse conditions. Although IL-2 and IL-15 do appear to be redundant, the question remains as to whether they have unique roles *in vivo*. And if they do, how is their biological specificity achieved? Two mechanisms that might contribute to the specificity IL-2 and IL-15 responses can readily be envisaged, the first involving ligand compartmentalization and the second regulation of IL-2Rα and IL-15Rα production. The available data suggest that both of these mechanisms may be relevant.

The distributions of IL-2-producing and IL-15-producing cells are very different [5]. IL-2 is made exclusively by activated T cells, whereas IL-15 is made by a wide variety of cells, including bone marrow stromal cells, gut and skin epithelia, macrophages and other cell types, but not by activated T cells [4,6,7]. These observations would suggest that IL-2 may have a limited role during T cell responses, whereas IL-15 may be involved more generally in multiple stages of lymphoid development and perhaps also in the function of non-lymphoid cells. IL-2Rα and IL-15Rα also appear to be expressed in different cell types. For example, activated T cells make abundant IL-2Rα, but exhibit very few IL-15-binding sites, whereas liver cells express a high level of IL-15Rα mRNA but no detectable IL-2Rα mRNA [2]. Together, these regulatory mechanisms could provide the means to delimit IL-2 and IL-15 responses, supporting the idea that IL-2 and IL-15 would not fully replace each other *in vivo*.

The generation of 'knockout' mice in which specific genes have been inactivated by targeted recombination has helped to answer the central question of the redundancy of IL-2 and IL-15. Although IL-2 has classically been defined as a T-cell growth factor, mice deficient in the IL-2 pathway — whether *IL-2*$^{-/-}$, *IL-2Rα*$^{-/-}$ or *IL-2Rβ*$^{-/-}$ homozygotes — have apparently normal thymic and peripheral T-cell development, ruling out an essential role for IL-2 signaling in these processes [3,8,9]. These mutant mice do, however, exhibit defects in peripheral lymphoid homeostasis and show evidence of inappropriate T-cell activation, lymphoid infiltration and autoimmunity [3, 8–10].

These data suggest that one of the unique roles for IL-2 *in-vivo* involves the termination of T-cell responses, as originally proposed by Lenardo (reviewed in [11]). In particular, IL-2 is suggested to be involved in a process of 'activation-induced cell death', in which repeated T-cell receptor triggering leads to a high local production of IL-2, upregulation of the apoptosis-inducing Fas/FasL system and subsequent apoptosis. How an absence of IL-2 perturbs this process is not clear, but it may modulate transduction of the 'death signal' via Fas. Two additional points should be emphasized. First, an absence of IL-2 appears to affect only thymus-derived T cells, as athymic (nude) mice deficient in IL-2 or γ_c are free from disease [8,10]. And second, the fact that this pathology is present in *IL-2*$^{-/-}$ and *IL-2Rα*$^{-/-}$ mutant mice clearly shows that IL-15, despite its broad distribution, cannot compensate for the absence of IL-2. Thus, IL-2 plays an essential role in peripheral T-cell homeostasis.

Extrathymic T-cell development has been best documented for gut intraepithelial lymphocytes, which comprise two distinct lineages, 'thymus-dependent' and 'thymus-independent' subsets, based on their different phenotypic and functional attributes (reviewed in [12]). The mechanisms that control thymus-dependent versus thymus-independent lymphoid differentiation are unclear and have been the subject of much debate. Recent studies of *IL-2Rβ*$^{-/-}$ mice by Suzuki *et al.* [13] suggest that IL-15 plays the paramount role in extrathymic lymphoid development. First, *IL-2Rβ*$^{-/-}$ mice completely lack NK cells, whereas these cells are present in *IL-2*$^{-/-}$ mice [8]. The NK defect in *IL-2Rβ*$^{-/-}$ mice was not completely unexpected in view of the many studies demonstrating the potent ability of IL-15 to support NK differentiation *in vitro* (see below). A second, more striking observation, was that *IL-2Rβ*$^{-/-}$ mice lack thymus-independent, but not thymus-dependent, gut intraepithelial lymphocytes. These observations clearly show that IL-15 has an essential *in vivo* role in extrathymic T and NK cell development.

How might IL-2 and IL-15 support extrathymic lymphoid development? And do IL-2 and IL-15 have distinct roles

in this process? Although IL-2 can support the development and proliferation of NK lineage cells *in vitro*, a number of observations suggest that IL-2 may not subserve this function *in vivo*. Thus, IL-2 is clearly not required for NK differentiation *in vivo*, as functional NK cells are present in *IL-2*$^{-/-}$ and *RAG*$^{-/-}$ mutant mice (which lack mature T cells, the only source of IL-2). Furthermore, IL-2 only stimulates NK generation *in vitro* at high concentrations [7], bringing into question whether these levels could be achieved physiologically *in vivo*. And if IL-2 were critical for NK development, activated T cells should be found at the site of NK cell genesis, in the bone marrow for example, but this has not been demonstrated.

IL-15, in contrast, appears more physiologically relevant to extrathymic lymphocyte development. Thus, IL-15 is a potent factor for NK differentiation, and can maintain NK cells *in vitro* at picomolar concentrations [14]. IL-15 can effectively substitute for the bone marrow stromal microenvironment for NK generation *in vitro* [15]. And as mentioned previously, IL-15 is produced by the correct cells and tissues — bone marrow stroma, macrophages, and gut and skin epithelia — to play a physiologically important role in NK cell development. In this context, the constitutive expression of IL-15 by macrophages provides an elegant mechanism for maintaining NK cells in their local microenvironment [6]; this would permit efficient NK cell–macrophage interactions, which are critical for the initiation of immune responses.

As for extrathymic T cell development, IL-15 has been shown to act as a potent growth factor for γδ T cells [16]. The finding that *IL-2Rβ*$^{-/-}$ mice lack the thymus-independent subset of gut intraepithelial lymphocytes, which comprise both γδ and αβ T cells [12], would again suggest that IL-15 has a major role in maintaining these cells *in vivo*. IL-15 mRNA has been detected in the gut epithelium of rats, whereas IL-2 mRNA has not been detected in this tissue [17]. Finally, IL-2-deficient mice show no defects in gut γδ T cell development, or γδ T cell development at other sites, for that matter [8]. Taken together, these observations support the view that IL-15 is essential for driving extrathymic T and NK cell development and for sustaining the innate immune system.

The observation that the IL-2 and IL-15 receptors have common subunits strongly suggested that these two cytokines have related functions *in vivo*. But although IL-2 and IL-15 are both essential for the maintenance of lymphoid cells, they act on and regulate distinct target cell populations, and appear to have inverse roles. Thus, IL-2 is required for homeostasis of thymic-derived T cells through termination of antigen-specific responses, whereas IL-15 appears essential for the survival and proliferation of extrathymic T and NK cells. This specificity is achieved by a combination of compartmentalization of cytokine production and differential expression of the IL-2Rα and IL-15Rα chains. The generation and analysis of IL-15 and IL-15Rα mutant mice will surely refute or confirm these hypotheses.

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